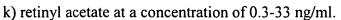
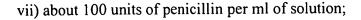
We claim:

- 1. A protein-free defined medium for culturing epidermal keratinocytes comprising:
 - a) N- (2-OH-ethyl-)piperazine-N -(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
 - b) sodium chloride at a concentration of 100-120 mM;
 - c) histidine at a concentration of 0.1-0.25 mM;
 - d) isoleucine at a concentration of 0.05-0.5 mM;
 - e) methionine at a concentration of 0.1-0.5 mM;
 - f) phenylalanine at a concentration of 0.1-0.5 mM;
 - g) tryptophan at a concentration of 0.05-0.5 mM;
 - h) tyrosine at a concentration of 0.1-0.5 mM; and
 - i) retinyl acetate at a concentration of 0.3-33 ng/ml.
- 2. A protein-free defined medium for culturing epidermal keratinocytes comprising:
 - a) N- (2-OH-ethyl-)piperazine-N'-(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
 - b) sodium chloride at a concentration of 100-120 mM;
 - c) calcium²⁺ ions at a concentration of 0.7-3.0 mM;
 - d) histidine at a concentration of 0.1-0.25 mM;
 - e) isoleucine at a concentration of 0.05-0.5 mM;
 - f) methionine at a concentration of 0.1-0.5 mM;
 - g) phenylalanine at a concentration of 0.1-0.5 mM;
 - h) tryptophan at a concentration of 0.05-0.5 mM;
 - i) tyrosine at a concentration of 0.1-0.5 mM;
 - j) β-transforming growth factor at a concentration of 3.0-30 ng/ml; and





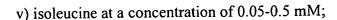
- 3. A protein-free defined medium for culturing epidermal keratinocytes comprising:
 - a) N-(2-OH-ethyl-)piperazine-N'-(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
 - b) sodium chloride at a concentration of 100-120 mM;
 - c) calcium.sup.2+ ions at a concentration of 0.7-3.0 mM;
 - d) histidine at a concentration of 0.1-0.25 mM;
 - e) isoleucine at a concentration of 0.05-0.5 mM;
 - f) methionine at a concentration of 0.1-0.5 mM;
 - g) phenylalanine at a concentration of 0.1-0.5 mM;
 - h) tryptophan at a concentration of 0.05-0.5 mM;
 - i) tyrosine at a concentration of 0.1-0.5 mM;
 - i) linoleic acid at a concentration of 1-15 ng/ml; and
 - k) retinyl acetate at a concentration of 0.3-33ng/ml.
- 4. A method for the formation of a histologically complete stratified epidermis comprising the steps of:
- a) isolation of basal stem cells from animal epithelium using a solution comprising:
 - i) glucose at a concentration of about 10 mM;
 - ii) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 16-22 mM;
 - iii) sodium chloride at a concentration of 90-140 mM;
 - iv) potassium chloride at a concentration of about 3 mM;
 - v) sodium orthophosphate (Na.sub.2 HPO.sub.4.7H.sub.2 O) at a concentration of 1 mM;
 - vi) phenol red at a concentration of 0.0033 mM;



- viii) about 100 units of streptomycin per ml of solution; and
- ix) trypsin at a concentration of 0.1%-0.2% w/v;
- b) recovering said isolated basal stem cells using a solution comprising:
 - i) glucose at a concentration of about 10 mM;
 - ii) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 16-22 mM;
 - iii) sodium chloride at a concentration of 90-140 mM;
 - iv) potassium chloride at a concentration of about 3 mM;
 - v) sodium orthophosphate (Na.sub.2 HPO.sub.4.7H.sub.2 O) at a concentration of 1 mM;
 - vi) phenol red at a concentration of 0.0033 mM;
 - vii) about 100 units of penicillin per ml of solution;
 - viii) about 100 units of streptomycin per ml of solution; and
 - ix) soy bean trypsin inhibitor at a concentration of 0.1%-1.0% w/v;
- c) culturing said isolated basal stem cells in a medium to form a confluent sheet of undifferentiated epithelial tissue, said medium comprising:
 - i) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14-22 mM;
 - ii) sodium chloride at a concentration of 100-120 mM;
 - iii) histidine at a concentration of 0.1-0.25 mM;
 - iv) isoleucine at a concentration of 0.05-0.5 mM;
 - v) methionine at a concentration of 0.1-0.5 mM;
 - vi) phenylalanine at a concentration of 0.1-0.5 mM;

- vii) tryptophan at a concentration of 0.05-0.5 mM;
- viii) tyrosine at a concentration of 0.1-0.5 mM; and
- ix) retinyl acetate at a concentration of 0.3-33 ng/ml;
- d) culturing said sheet of undifferentiated epithelial tissue in a differentiation medium to form a sheet of differentiated and stratified tissue, said differentiation medium comprising:
 - i) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14-22 mM;
 - ii) sodium chloride at a concentration of 100-120 mM;
 - iii) calcium²⁺ ions at a concentration of 0.7-3.0 mM;
 - iv) histidine at a concentration of 0.1-0.25 mM;
 - v) isoleucine at a concentration of 0.1-05 mM;
 - vi) methionine at a concentration of 0.1-0.5 mM;
 - vii) phenylalanine at a concentration of 0.1-0.5 mM;
 - viii) tryptophan at a concentration of 0.05-0.5 mM;
 - ix) tyrosine at a concentration of 0.1-0.5 mM;
 - x) β-transforming growth factor at a concentration of 3.0-30 ng/ml; and
 - xi) retinyl acetate at a concentration of 0.3-33 ng/ml; and
- e) culturing said differentiated and stratified tissue in a cornification medium to form a cornified epithelium, said cornification medium comprising:
 - i) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14- 22 mM;
 - ii) sodium chloride at a concentration of 100-120 mM;
 - iii) calcium.sup.2+ ions at a concentration of 0.7-3.0 mM;
 - iv) histidine at a concentration of 0.1-0.25 mM;





- vi) methionine at a concentration of 0.1-0.5 mM;
- vii) phenylalanine at a concentration of 0.1-0.5 mM;
- viii) tryptophan at a concentration of 0.05-0.5 mM;
- ix) tyrosine at a concentration of 0.1-0.5 mM;
- x) linoleic acid at a concentration of 1-15 ng/ml;
- xi) retinyl acetate at a concentration of 0.3-33 ng/ml.
- 5. The method according to claim 4 wherein said histologically complete epidermis is human epidermis.
- 6. The method according to claim 4, further comprising the step of using serum or tissue extract or animal derived factors or other xenobiotics in combination with Ca²⁺ ions (1-2 mM) in order to form a stratified keratinizing epithelium.